

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 January 2006 (12.01.2006)

PCT

(10) International Publication Number
WO 2006/004267 A1

(51) International Patent Classification⁷: **C12Q 1/68**
(21) International Application Number:
PCT/KR2005/000889

(22) International Filing Date: 25 March 2005 (25.03.2005)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
10-2004-0020444 25 March 2004 (25.03.2004) KR

(71) Applicant (for all designated States except US):
BIONEER CORPORATION [KR/KR]; 49-3,
Munpyeong-dong, Daedeok-gu, Daejeon 306-220 (KR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **PARK, Han Oh**
[KR/KR]; 208dong 601ho, Expo Apt., Jeonmin-dong,
Youseong-gu, Daejeon 305-761 (KR). **KIM, Hyun**
Bae [KR/KR]; 205dong 802ho, Songlim Maeul Apt.,
Haki-dong, Youseong-gu, Daejeon 305-358 (KR). **CHI,**
Sung Min [KR/KR]; 102dong 203ho, Gyeongnam Apt.,
Eomsa-ri, Duma-Myeon, Gyeryong-si, Chungcheong-
nam-do 320-915 (KR).

(74) Agent: **SEO, Keun Bok**; 1003ho, Sungji Heights III Bldg.,
642-6, Yeoksam-dong, Gangnam-gu, Seoul 135-080 (KR).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DETECTION METHOD OF DNA AMPLIFICATION USING PROBE LABELED WITH INTERCALATING DYE

(57) Abstract: The present invention relates to a detection method of nucleic acid amplification using probe labeled with intercalating dye. More particularly, the present invention is directed to a real-time detection method of nucleic acid amplification, comprising the steps of i) producing an aqueous buffer which contains a nucleic acid, a pair of primers for amplification of said nucleic acid, a fluorescent probe wherein a fluorescent dye of which intensity of fluorescence is varied when the dye is intercalated into a double-stranded nucleic acid, is connected with an oligonucleotide of which base sequence is complementary with at least a part of said nucleic acid, four(4) kinds of nucleotides and DNA polymerase; ii) denaturing said doublestranded nucleic acid into single strands by heating the aqueous buffer prepared in step i) up to 93°C to 96°C; iii) annealing said pair of primers with said single strand by cooling the solution obtained in step ii) up to 50°C to 57°C; iv) replicating said single-stranded nucleic acid by heating the solution obtained from step iii) up to 70°C to 74°C; v) denaturing said replicated nucleic acid into single strands by heating the solution obtained in step iv) up to 93°C to 96°C; vi) annealing said fluorescent probe with said single-stranded nucleic acid by cooling the solution obtained in step v up to 50°C to 57°C; vii) measuring an intensity of the fluorescence emitted from the solution obtained in step vi); and viii) repeating more than one steps iv) through vii).

WO 2006/004267 A1